REMARKS

New claims 47-57 have been added. Support for these new claims can be found at page 6, line 15 through page 7, line 10.

The Examiner has made the Restriction Requirement final. The withdrawn claims, Claims 20-46, have been canceled without prejudice or disclaimer of the subject matter claimed therein.

The Examiner has rejected Claims 1-6 and 14 under 35 USC 102(b) as being anticipated by Gudin et al. (U.S. Patent No. 5,179,021).

The Examiner has rejected Claims 1, 7-10 and 12-19 under 35 USC 103(a) as being unpatentable over Gudin et al. in view of Barclay (US Patent No. 5,130,242).

The Examiner has rejected Claims 1, 7-9 and 11 under 35 USC 103(a) as being unpatentable over Gudin et al. in view of Wagner (US Patent No. 5,720,456).

The Examiner's rejections of Claims 1-19 under 35 USC 102(b) and/or 35 USC 103(a) are respectively traversed for the following reasons.

The present invention is directed to a method for obtaining lipids from microorganisms. Each of the pending claims is directed to the recovery of such lipids. In particular, the claims are directed to an efficient and effective method for obtaining such lipids from microorganisms using phase separation.

In the past, such lipids were often recovered using solvent extraction, and, in particular, employing a nonpolar solvent such as hexane. The present invention has a number of advantages over such prior art solvent extraction techniques, as are discussed in the present application.

The present invention is patentable over Gudin et al. for a number of important reasons.

Gudin et al. teach a straightforward solvent extraction process (see column 2, lines 51-53;

column 4, lines 3-21 and column 5, line 56-column 6, line 11). In a solvent extraction process, the desired product (in the case of Gudin et al., antioxidants) preferentially migrates to the desired solvent phase and can be separated and recovered. In other words, the solvent extraction process of Gudin et al. results in a phase containing the cellular debris and one or two phases comprising the added solvent(s) and the product(s) which preferentially migrate to the solvent(s). On the other hand, the present invention involves a straightforward phase separation. The desired product (lipid) is efficiently and effectively separated from the fermentation broth, e.g., by centrifugation. While additives, such as alcohols, may aid in the separation, the present invention is a phase separation, not a solvent extraction. As a result, the cellular debris is in the aqueous phase, and the desired lipid product is in its own lipid-rich phase, rather than dissolved or dispersed in an added solvent phase. The lipid-rich phase produced by the present invention can be subjected to further refining and/or polishing. The present process has a number of important advantages, including simplicity, efficiency and effectiveness. For example, the fermentation broth can be treated directly, without drying the microorganisms.

Gudin et al. is <u>not</u> directed primarily to obtaining lipids. Instead, Gudin et al. teaches a solvent extraction process for recovering antioxidants, of which some antioxidants are lipid-soluble and some are water-soluble. The lipid-soluble antioxidants listed by Gudin et al. are alpha, beta, and gamma tocopherols; the water-soluble antioxidants listed by Gudin et al. are vitamin C and superoxide-dismutase enzymes (SOD). As disclosed in column 1, lines 13-28 of Gudin et al., SOD are metal enzymes having two peptide subunits linked to one another, i.e., the SOD are proteins. The only antioxidants that the process described by Gudin et al. removes without significant quantities of organic solvent are the water-soluble antioxidants and specifically only the SOD (column 4, lines 3-

20; column 5, lines 35, 56-61). Therefore, one skilled in the art would not look to Gudin et al. for a teaching regarding how to recover lipids from a microorganism without the use of any significant amount of organic solvent.

The Gudin et al. patent teaches about the recovery of antioxidants -- compounds that occur at very low concentrations in the cells (e.g., less than 1% of dry weight; see Gudin et al., column 7, lines 45-52). The present application teaches how to recover a product (lipids) that can occur in the cells at greater than 30%, technically a very different problem. The Gudin et al. process requires preconcentration of the cells (by centrifugation or rotary drum drying) prior to recovery of the lipid fraction. Such preconcentration is not necessary in the present process, and thus this costly and inefficient step may be avoided in the present process.

The Examiner admits that Gudin et al. do not specifically teach adding a base selected from hydroxides, carbonates, bicarbonates or mixtures thereof to adjust pH. However, the Examiner has stated that Barclay teaches that growth of the strains results in a fermentation broth that is more alkaline and the preferred range of pH is 5.5-8.5, citing column 9, lines 34-41 of Barclay. While it is true that Barclay teaches growing cells at pH 4-8.5 to maximize production, that is very different than the pH's achieved when adding a base to "hydrolyze a portion of the proteins". The pH employed to hydrolyze a portion of the proteins is above pH 9, and preferably greater than pH 10 or pH 11, which is above the pH where the vast majority of microorganisms can grow or survive (in part due to the hydrolysis of proteins necessary for life and interruption of biochemical processes). The present application teaches destruction of the proteins because the inventors identified them as key components maintaining the emulsion of oil and water that hinders recovery of the lipids. On the other hand, Gudin et al. are trying to recover the proteins. SOD is an enzyme (protein) so Gudin

et al. use conditions that protect the proteins (e.g., 4°C temperature, ammonium sulfate precipitation) and do not denature or hydrolyze them. The conditions of the present invention, focusing on an economic method for recovery of the lipids, hydrolyzes and denatures the proteins. Step (c) of the Gudin et al. process calls for "dissolving" the cells (after preconcentrating them via centrifugation or vacuum drum filtering). However, this step is really a step where the cells are resuspended to a desired concentration before running them through the homogenizer. On the other hand, the present invention actually teaches true dissolving/lysing of the cells, and without a preconcentration step. Therefore, the Gudin et al. and Barclay references do not render Claim 11 obvious.

Because the present process does not involve solvent extraction, organic solvents are not required. If any organic solvents are present, they function as a process aid, preferably at a concentration less than 5%. New claims 47-50 and 52 have been added to particularly point out this aspect of the present invention. The Gudin et al. process is very standard regarding solvent use and those skilled in the art would know that it would require about 2 parts solvent to 1 part culture media to have effective recoveries -- this is 100-200% compared to less than 5%, again demonstrating that the present process is efficient and economical, and results in products having added value due to solvent-free processing.

There are further specific differences between the claims and the process taught by Gudin et al. For example, Claim 11 requires heating the cells to at least 50C, while Gudin et al. teach a temperature of 4°C (column 4 lines 23-27). Gudin et al. heat the cells briefly to 40C to "permeablize" the cells and let the water-soluble antioxidants leak out into the culture medium where they can be concentrated, purified and recovered. Gudin et al. then centrifuge/rotary drum filter to recover the cells and process them for the lipid related antioxidants. On the other hand, Claim 11

claims heating above 50C to lyse the lipid rich cells -- and the cells do not have to be recovered. Claims 10 and 57 require that at least a portion of the proteinaceous compounds in the fermentation broth be solubilized, which is not specifically taught by Gudin et al. In Claims 51and 57, the process is conducted without drying the cells and in Claims 53-57, the microorganisms contain from at least 10%, up to at least 50%, entrained water. On the other and, Gudin et al. employ a rotary filter (#7) to dewater the cells (see column 5, lines 25-49 and Figure 1).

As a result of the substantial differences between the cited art and the present invention, the claimed process is much simpler, less expensive and more efficient than the cited art, and in particular, Gudin et al. As a simple example, referring to Figure 1 in Gudin et al., the present claimed process only requires the culture vessel and the centrifuge (#4 and #18), and the other major process steps and equipment illustrated in Figure 1 are not required.

The other references cited by the Examiner (Barclay and/or Wagner) do not overcome the deficiencies and inadequacies of Gudin et al. as a reference.

It is respectfully submitted that all of Claims 1-19 and 47-57 are in condition for allowance, and that the present case should be passed to issue. In the event that the Examiner has any questions or concerns regarding the allowability of any claim, please consider this a provisional request for an Examiner's interview and please contact the undersigned attorney at 303-863-2973.

Attached hereto is a marked up version of the changes made to the claims by the current amendment. The attached page is captioned "Marked-Up Version Showing Amendments."

It is not believed any fees are due, however, in the event any fees are due, please debit any underpayment to Deposit Account No. 19-1970.

Respectfully submitted,

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Date: February 5, 2007

New Claims 47-57 have been added.